J. Parasitol., 88(5), 2002, pp. 1038–1040

Rotifers Ingest *Giardia* Cysts

J. M. Trout, E. J. Walsh*, and R. Fayer, Animal Waste Pathogen Laboratory, Animal and Natural Resources Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705. *University of Texas El Paso, Department of Biology, El Paso, Texas. *e-mail: jtrout@anri.barc.usda.gov*

ABSTRACT: Seven species of rotifers representing 6 genera, *Epiphanes*, *Plationus*, *Asplanchna*, *Philodina* species A, *Philodina* species B, *Platyias*, and *Brachionus*, were exposed to *Giardia* cysts isolated from the feces of experimentally infected holstein calves. *Giardia* cysts were prestained with a fluorescein isothiocyanate–conjugated monoclonal antibody and mixed with viable rotifers on 3-well Teflon-coated microscope slides. Organisms were observed with phase-contrast, differential interference contrast, and fluorescence microscopy. Five rotifer species, *Epiphanes brachionus*, *Plationus patulus*, *Philodina* (both A and B), and *Platyias quadricornis*, ingested varying numbers of cysts, which were retained within the rotifers' bodies throughout the observation period. Rotifer ingestion of *Giardia* cysts may represent a means of reducing water contamination.

Rotifers are a large group of free-living microscopic organisms consisting of more than 1,850 species that may be free-swimming, sessile, or benthic (Segers, 2002). They are found in diverse locations such as freshwater lakes and ponds, brackish and salt water, and virtually any other place where water accumulates, such as puddles, damp soil, and even birdbaths (Nogrady et al., 1993). Rotifers thrive in highly eutrophic environments such as wastewater and domestic sewage, which are not conducive to the survival of their major predators, copepods. Ranging in length from 50–2,000 μm, rotifers possess a ciliated corona at their anterior end that is used for locomotion and food gathering (Wallace and Snell, 2001). Although the structure of the corona varies widely among species, the beating cilia sweep water and suspended particles toward a central oral cavity where a second set of cilia reject particles unsuitable for ingestion (Gilbert and Starkweather, 1977, 1978; Wallace and Snell, 2001). Particles that enter the oral cavity pass into the mastax, an organ with chitinous plates that chops food and pushes it into the esophagus. From the esophagus, particles move into the stomach and then into an intestinal tube (Nogrady et al., 1993). One rotifer species was reported to have 5 digestive glands (Schramm, 1978). Digestive enzymes, such as glycosidases and endoglucanases, were identified in homogenates from another species (Kuhle and Kleinow, 1990; Chun et al., 1997).

Giardia includes a number of species capable of causing infection in a variety of vertebrate hosts, including humans. Human giardiasis is the most commonly diagnosed protozoan intestinal disease worldwide. Infection is often accompanied by intestinal distress, diarrhea, malabsorption, and impaired growth (Farthing, 1994). The infective stage, an environmentally resistant cyst, can be found in surface waters throughout the world. *Giardia* spp. cysts are similar in size to the food particles ingested by rotifers, and because rotifers were reported to ingest oocysts of the protozoan parasite *Cryptosporidium parvum* (Fayer et al., 2000), the present study was undertaken to determine if rotifers would also ingest *Giardia* spp. cysts. If rotifers do indeed ingest cysts, this could provide clues to the environmental fate of cysts and suggest potential biological control strategies for reducing *Giardia* spp. contamination of surface water.

Giardia spp. cysts were isolated from the feces of experimentally infected neonatal holstein calves and used within 2 wk. Briefly, feces were passed through a series of screens of increasingly finer mesh and further purified by centrifugation over a discontinuous CsCl gradient (Kilani and Sekla, 1987). This procedure is routinely used in our laboratory to isolate cysts from feces and results in a highly purified, viable population of cysts that are then used to infect subsequent calves to maintain continuous propagation of this *Giardia* isolate. Purified cysts were washed extensively to remove residual CsCl and were then labeled with a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) (Mer*IF*luor®, Meridian Biosciences, Inc., Cincinnati, Ohio) by suspending them in 200 μl of the detection reagent. After 30 min of incubation at room temperature, cysts were washed thoroughly to remove excess antibody and then resuspended in springwater at a concentration of 4,000 cysts/μl. Different rotifer species were either collected from nature or purchased (Carolina Biological Supply Co., Burlington, North Carolina) (Table I). The rotifers were maintained in 100-ml polyethlyene screw-capped jars and were fed approximately every 2 days with a mixture of *Chlamydomonas reinhardtii* (UTEX-90) and *Ankistrodesmus falcatus* (UTEX-749). No effort was made to starve the rotifers before testing. Cultures were monitored daily using an Olympus CK2 inverted microscope equipped with both 10× and 20× phase-contrast objectives. To obtain rotifers for testing, 5–15 μl was aspirated with a pipet from heavily populated areas of the cultures, or in the case of sparsely populated cultures, individual organisms were located with the inverted microscope and removed using a pipet. Approximately 5-μl aliquots containing rotifers were placed into a 11-mm-diameter well of a heavy teflon®–coated 3-well slide (Cell-Line, Erie Scientific, Portsmouth, New Hampshire); 3–6 slides (9–18 wells) were prepared for each rotifer species tested. The presence of rotifers in the wells was verified by microscopic observation. Two microliters of the prestained *Giardia* cyst suspension (8,000 cysts) was added to each well, with 1 well for each rotifer species receiving no cysts to serve as a negative control. After the addition of the cysts, each slide was allowed to stand undisturbed for 5 min, gently covered with a 22- × 50-mm glass coverslip, and observed for 15–20 min using a Zeiss Axioskop 20 microscope equipped with epifluorescence and a Texas Red–FITC dual wavelength filter and DIC optics. Digital images were obtained with a Spot RT Slider digital camera and Spot version 3.2.4 software (Diagnostic Instruments, Inc., Sterling Heights, Michigan).

TABLE I. Sources of rotifers used in the present study.

Species	Source	Location	Collection permit
Asplanchna sieboldi	Eastwood City Park	El Paso Co., Texas	NA
Brachionus plicatilis (brackish water species)	Commercial*	—	NA
Epiphanes brachionus	Hueco Tanks State Historical Park	El Paso Co., Texas	#66-99
Philodina species A†	Cattail Springs, Big Bend National Park	Brewster Co., Texas	BIBE-2001-SCI-0058
Philodina species B	Commercial*	—	NA
Plationus patulus	Pond, New Mexico, Highway 180	Dona Ana Co., New Mexico	NA
Platyias quadricornis	Beaver Pond, Rio Grande Village, Big Bend National Park	Brewster Co., Texas	BIBE-2001-SCI-0058

* Carolina Biological Supply Co., Burlington, North Carolina. NA, not applicable.
† Subsequently identified as *Philodina megalotrocha*.

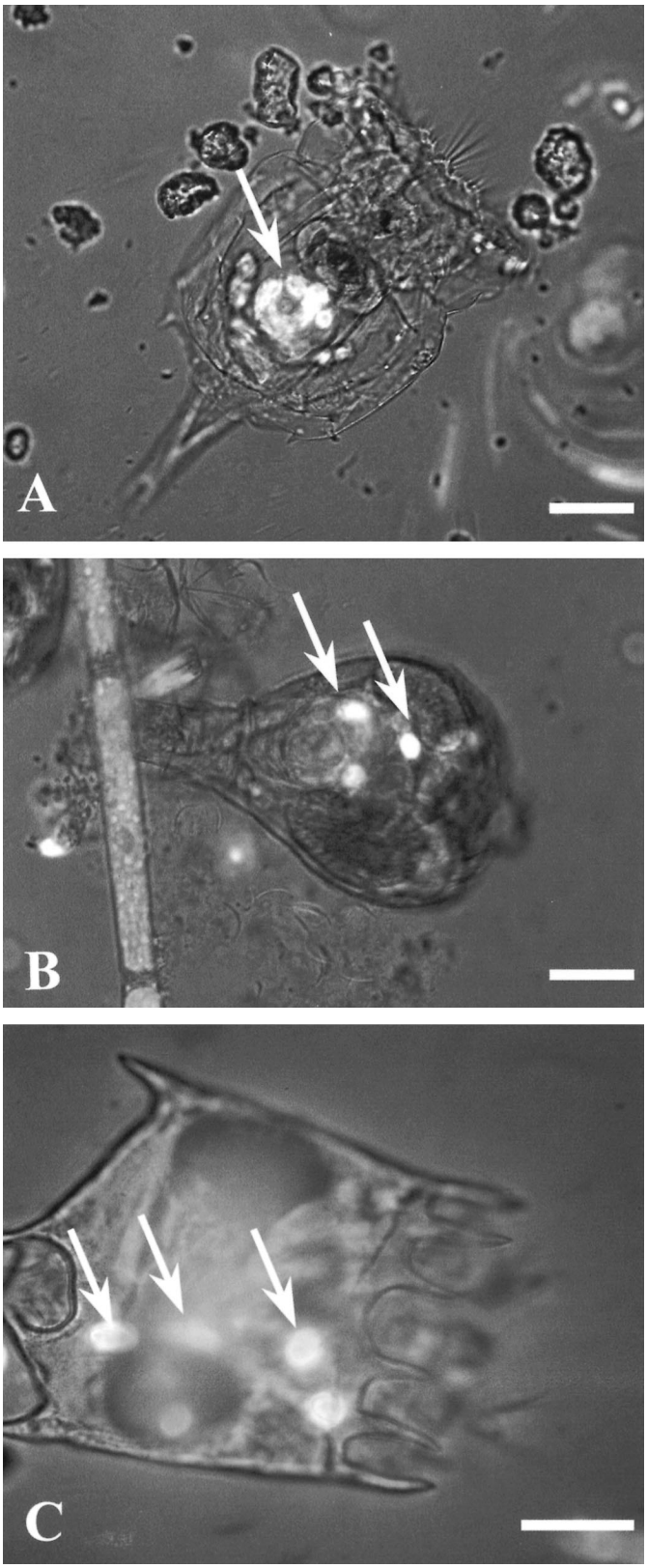

FIGURE 1. Digital photomicrographs of 3 rotifers with ingested *Giardia* cysts taken under epifluorescence illumination. Arrows point to a cluster of cysts (**A**) and individual cysts (**B, C**). **A.** *Epiphanes brachionus*. **B.** *Philodina* species A. **C.** *Plationus patulus*. Bar = 45 μm.

Of the 6 genera of rotifers tested (Table I), 5 species ingested *Giardia* cysts. Quantitation within and between genera, however, was difficult; the cysts were relatively small, whereas the rotifers were often relatively large and highly mobile, thus requiring continuous focusing through the body of the rotifer as well as 2-dimensional microscope stage movements. Because of the difficulty previously experienced in observing and photographing unlabeled oocysts of *C. parvum* ingested by rotifers (Fayer et al., 2000), only *Giardia* sp. cysts labeled with FITC-conjugated mAb were used in the present study. It is not known if this labeling might enhance or reduce the ingestion of cysts, but both labeled and unlabeled *C. parvum* oocysts were observed previously as being ingested by rotifers (Fayer et al., 2000).

Epiphanes brachionus (Fig. 1A) and *Philodina* species A (Fig. 1B) consistently ingested the most cysts (6–10 and 6–12 cysts/organism, respectively). *Plationus patulus* (Fig. 1C), *Philodina* species B, and *Platyias quadricornis* ingested fewer cysts (1–6, 0–1, and 2–8 cysts/organism, respectively), whereas *Asplanchna sieboldi* and *Brachionus plicatilis* were not observed to ingest any cysts. Precise numbers of cysts ingested could not be accurately determined because of the movement of the rotifers as well as their body contents, and previous attempts at fixation were problematic (Fayer et al., 2000). Overall, the numbers of cysts ingested tended to be lower than the numbers of *C. parvum* oocysts previously observed to be ingested by rotifers (Fayer et al., 2000); however, *Giardia* sp. cysts are 3–4× larger than the oocysts and may possibly have different surface characteristics. Interestingly, *E. brachionus*, which in this study readily ingested *Giardia* cysts, ingested few *C. parvum* oocysts in the previous study. Likewise, *Philodina* species B ingested few *Giardia* cysts in this study and yet readily ingested *C. parvum* oocysts in the previous study (Fayer et al., 2000). This could indicate a variable preference in food particle size and characteristics between genera of rotifers or, as illustrated by the differences in cyst ingestion between *Philodina* species A and B, even between members of the same species.

Reports indicate that gut transit time in rotifers varies with species and external conditions, ranging from 2–3 min to 15–20 min (Resvoi, 1926) with an average of about 20 min in *B. calcyfluorus* (Starkweather and Gilbert, 1977). In the study involving *C. parvum* oocysts, *E. brachionus* and *Euchlanis triquetra* were observed to excrete boluses of oocysts mixed with other material within 15 min after ingestion. None of the rotifers in the present study was observed to excrete *Giardia* cysts but rather retained cysts within the bodies throughout the 15- to 20-min observation period (20–25 min after the addition of the cysts). Attempts at longer observation times resulted in decreased rotifer viability. Sometimes, a diffuse green glow was observed within the rotifers, suggesting that the FITC-labeled mAb may have been dissociating from the surface to the cysts; stained material also appeared to dissociate in the *C. parvum* study. Digestion in rotifers is reported to take 15–20 min, and digestive enzymes appear to act mainly on carbohydrates (Kuhle and Kleinow, 1990; Chun et al., 1997); however, it could not be determined if cysts were actually digested or if gut conditions simply disrupted the interaction between the mAb and the epitopes on the cyst surface.

The current observations were made on rotifers and cysts in an artificial setting. It is unknown whether rotifers ingest *Giardia* sp. cysts in a natural environment. But rotifers thrive in eutrophic environments such as animal waste lagoons, wastewater facilities, and other locations where *Giardia* sp. cysts would most likely enter the environment. Although it could not be determined if the rotifers damaged or degraded the cysts after ingestion, the simple fact that they were retained within the organisms could help to limit environmental dispersion of the cysts. Certainly, further experiments in a more natural environment are called for to help determine if rotifers have the potential for use as a biocontrol agent for *Giardia* sp. and possibly other protozoan parasites.

The authors wish to thank Kristie Ludwig for her expert technical assistance.

LITERATURE CITED

CHUN, C. Z., S. B. HUR, AND Y. T. KIM. 1997. Purification and characterization of an endoglucanase from the marine rotifer *Brachionus plicatilis*. Biochemistry and Molecular Biology International **43:** 241–249.

FARTHING, M. J. G. 1994. Giardiasis as a disease. *In* Giardia: From

molecules to disease, R. C. A. Thompson, J. A. Reynoldson, and A. J. Lymbery (eds.). CAB International, Wallingford, U.K., p. 15–37.

FAYER, R., J. M. TROUT, E. WALSH, AND R. COLE. 2000. Rotifers ingest oocysts of *Cryptosporidium parvum*. Journal of Eukaryotic Microbiology **47:** 161–163.

GILBERT, J. J., AND P. L. STARKWEATHER. 1977. Feeding in the rotifer *Brachionus calyciflorus*. I. Regulatory mechanisms. Oecologia **28:** 125–131.

———, AND ———. 1978. Feeding in the rotifer *Brachionus calyciflorus*. III. Direct observations on the effects of food type, food density, and starvation on the incidence of psuedotrochal screening. Internationale Vereinigung fuer Theoretische und Angewandlte Limnologie Verhandlungen **20:** 2382–2388.

KILANI, R. T., AND L. SEKLA. 1987. Purification of Cryptosporidium oocysts and sporozoites by cesium chloride and percoll gradients. American Journal of Tropical Medicine and Hygiene **36:** 505–508.

KUHLE, K., AND W. KLEINOW. 1990. Glycosidases in *Brachionus plicatilis* (Rotifera). Comparative Biochemistry and Physiology. B: Comparative Biochemistry **95:** 393–402.

NOGRADY, T., R. L. WALLACE, AND T. W. SNELL. 1993. Rotifera. Vol. 1: Biology, ecology, and systematics. *In* Guides to the identification of the microinvertebrates of the continental waters of the world, H. J. Dumont (ed.). SPB Academic Publishing, The Hague, The Netherlands, p. 1–142.

RESVOI, P. 1926. Observations on the feeding of rotifers. Travaux de la Societe des Naturalistes de Leningrad **56:** 73–89. [In Russian.]

SCHRAMM, U. 1978. Studies of the ultrastructure of the rotifer *Habrotrocha rosa* Donner (Aschelminthes). The alimentary tract. Cell and Tissue Research **189:** 525–535.

SEGERS, H. 2002. Contributions to the nomenclature of Rotifera: Annotated checklist of valid family- and genus-group names. Journal of Natural History **36:** 631–640.

STARKWEATHER, P. L., AND J. J. GILBERT. 1977. Radiotracer determination of feeding in *Brachionus*: The importance of gut passage times. Archiv Fur Hydrobiologie Beiheft **8:** 261–263.

WALLACE, R. L., AND T. W. SNELL. 2001. Rotifera. Chapter 8. *In* Ecology and classification of North American freshwater invertebrates, 2nd ed., J. Thorp and A. Covich (eds.). Academic Press, New York, p. 195–254.